ON THE LUNAR ORIENTATION OF SANDHOPPERS (AMPHIPODA TALITRIDAE)

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The sandhopper, *Talitrus saltator,* possesses a mechanism of astronomical orientation which enables it to return by the shortest route to its habitat (the moist area of the beach) when it has been taken away from it. During the day, the animals use the position of the sun to orient (Pardi and Papi, 1952, 1953), while during the night they are able to orient by means of the moon (Papi and Pardi, 1953, 1954, 1959; Papi, 1960). The oriented escape occurs when the animals are placed in unfavorable surrounding conditions. For example, a very low degree of relative humidity or an elevated temperature in the surroundings can act as releasing stimuli. The statistical study of oriented behavior during the return to the sea has been carried out by placing the animals in a concave glass observation chamber on whose dry walls the sandhoppers climb in the direction of the sea.

As early as 1953 we were able to establish that the solar orientation is controlled by an endogenous rhythm and this was confirmed in further research (Papi, 1955; Pardi and Grassi, 1955). More recent research on the lunar orientation (Papi and Pardi, 1959) indicated that a second rhythm, independent of the solar one, may control the angle of orientation with the moon. In fact, even animals kept in constant darkness from sunset onwards or from the new moon preceding the night of the experiment are, for the most part, capable of correct orientation.

As soon as animals are introduced into the observation chamber they orient toward the moon. Only on particularly warm dry nights do the animals change spontaneously from this positive phototactic behavior to a correct orientation toward the sea. It is therefore necessary to heat the observation chamber or to dehydrate the air inside in order to bring about an oriented escape. In the experiments reported in 1959, as well as those in this report, we have always used the method of heating the base of the observation chamber.

Enright has recently (1961) published a paper on the lunar orientation of *Orchestoidea corniculata* in which he arrived at conclusions which differed in part from our own. Enright experimented with animals held in three different sets of circumstances: (A) those kept in constant darkness for ten or more hours before the experiment ("constant darkness"); (B) animals placed in constant darkness at the time of collection and re-exposed to natural light one hour prior to sunset and thus held until the moment of the experiment ("natural light"); (C) animals treated like the former but redarkened two hours after moonrise ("redarkened").

While in the "natural light" and "redarkened" animals Enright found a correct orientation, in the "constant darkness" animals there seemed to be only the tendency to assume a constant angle with the moon, regardless of the lunar stage or position. From this evidence the author drew the conclusion that the existence of a continuously operating endogenous lunar periodicity, which we postulated for *Talitrus,* is not admissible for *Orchestoidea.* Enright suggested that the correct orientation of the "natural light" and the "redarkened" animals could be explained (p. 155) as "a single-cycle night-time orientation rhythm re-initiated by the appropriate stimuli each night."

Concerning the methods used by Enright, we may note that he neither heated the observation chamber nor dehydrated the air inside. In addition, he has observed that in his experimental conditions the repeated use of photo-bulb flashes at short intervals, for making photographic recordings, produced a considerable change in the angle of orientation and in the dispersion of the animals. In the first series of experiments, in each of which three photographs were made, the angle of orientation with the moon tended generally to increase, and there was a similar increase in the dispersion. In two successive experiments, in which ten photographs were made, the author observed instead that (p. 152) "the ultimate angle of orientation with the moon . . . was much smaller than the initial angle."

Since the results of Enright's "constant darkness" animals contrast with those which we obtained from the sandhoppers maintained in darkness from sunset or from the new moon preceding the night of the experiment, and in view of his results on the effects of photo-bulb flashes, which throws doubt upon the validity of the method we employed, we wished to perform a new series of experiments on *Talitrus saltator.*

Materials and Methods

We used *Talitrus saltator* Montagu from the beach at Castiglione della Pescaia (Grosseto) where the theoretical line of escape is 201°. The apparatus was the same as that used for the experiments reported in 1959. The observation chamber was constantly heated, so that the air temperature of the interior was around 22° C.

For the series of experiments, 1, 3, and 4, the animals had been collected the morning before the night of the experiment, between 1000 and 1115. Only for the series of experiments 2 was the collection made in the afternoon (1800–1845), about one hour before sunset. After collection the animals were kept in darkness, in jars containing moist sand, until the moment of their introduction into the observation chamber. The introduction always took place in moonlight and without the aid of artificial light. After five minutes the first photograph was taken, and then eleven others were made at one-minute intervals, except in one case where only ten followed (experiment 4b).

The experiments were performed with a perfectly clear sky on four nights in the summer of 1961. On each night two to four experiments were carried out with as many different groups of animals. For each experiment a variable number of animals (from 16 to over 50) was used, but the number of positions totally recorded (see Table I) is not always a multiple of the number of photographs taken, because of the fact that on some photographs some individuals were not visible (perhaps because the photographs were taken while the animals were jumping). Thus, we obtained single distributions from individual photographs and were able to calculate accumulated distribution.

For each distribution (single or accumulated), we calculated the angular value of the average orientation direction (RD) and the length of the resultant vector, which measures the degree of scatter (OR) (see Pardi and Papi, 1953, p. 463, footnote 1). We have also calculated the average RD and OR for the single distributions, but they are so close to the RD and OR of the accumulated distribution (a maximum difference of ±1° and of ±0.01, respectively), that we have not considered it necessary to report them.

The expected, or theoretical, angle of orientation is the horizontal angle between the moon and the direction to the sea. The observed angle of orientation is the horizontal angle between the average direction of orientation (RD) and the moon. The position of the moon at the mid-point of each experiment was used. In determining these angles we have used for convenience a notation of positive when the moon was to the animal's left and negative when it was to their right.

TABLE I

Orientation of Talitrus saltator with moon

1. Reference number	2. Date	3. Time	4. Lunar stage: days	5. Lunar azimuth	6. No. of recorded positions	7. RD*	8. RD_1**–RD	9. Observed angle with moon	10. Theoretical angle with moon	11. Difference, 9–10	12. Vector length (OR)
1a	26 August	$21^h53^m30^s$	16	128°	192	177°	+ 2°	+49°	+73°	−24°	0.78
1b	27 August	$01^h37^m30^s$	16	189°	252	178°	− 1°	−11°	+12°	−23°	0.79
1c	27 August	$04^h10^m30^s$	16	231°	228	205°	− 5°	−26°	−30°	+ 4°	0.80
2a	27 July	$22^h06^m30^s$	16	144°	352	165°	+ 2°	+21°	+57°	−36°	0.74
2b	28 July	$00^h25^m30^s$	16	179°	341	171°	− 3°	− 8°	+22°	−30°	0.66
3a	28 July	$22^h30^m30^s$	17	134°	240	153°	−15°	+19°	+67°	−48°	0.80
3b	29 July	$02^h58^m30^s$	17	204°	191	197°	0°	− 7°	− 3°	− 4°	0.95
4a	3 July	$01^h15^m30^s$	20	126°	300	157°	+ 8°	+31°	+75°	−44°	0.89
4b	3 July	$02^h06^m—^s$	20	137°	585	150°	+ 6°	+13°	+64°	−51°	0.61
4c	3 July	$02^h50^m30^s$	20	148°	192	166°	+ 4°	+18°	+35°	−35°	0.85
4d	3 July	$03^h23^m30^s$	20	157°	299	168°	+12°	+11°	+44°	−33°	0.68

* RD: The resultant direction of orientation calculated by means of the complete distribution.
** RD_1: The resultant direction of orientation calculated from the registered distribution of the first photograph.

RESULTS

1. Effects of the photo-bulb flash

In each experiment, the variations of the RD of the OR in the single distributions, as compared with the RD and the OR of the accumulated distributions, are represented in Figures 1 and 2. The oscillations of RD are modest, with a difference between maximum and minimum at the most less than 20°. In one case (3a)

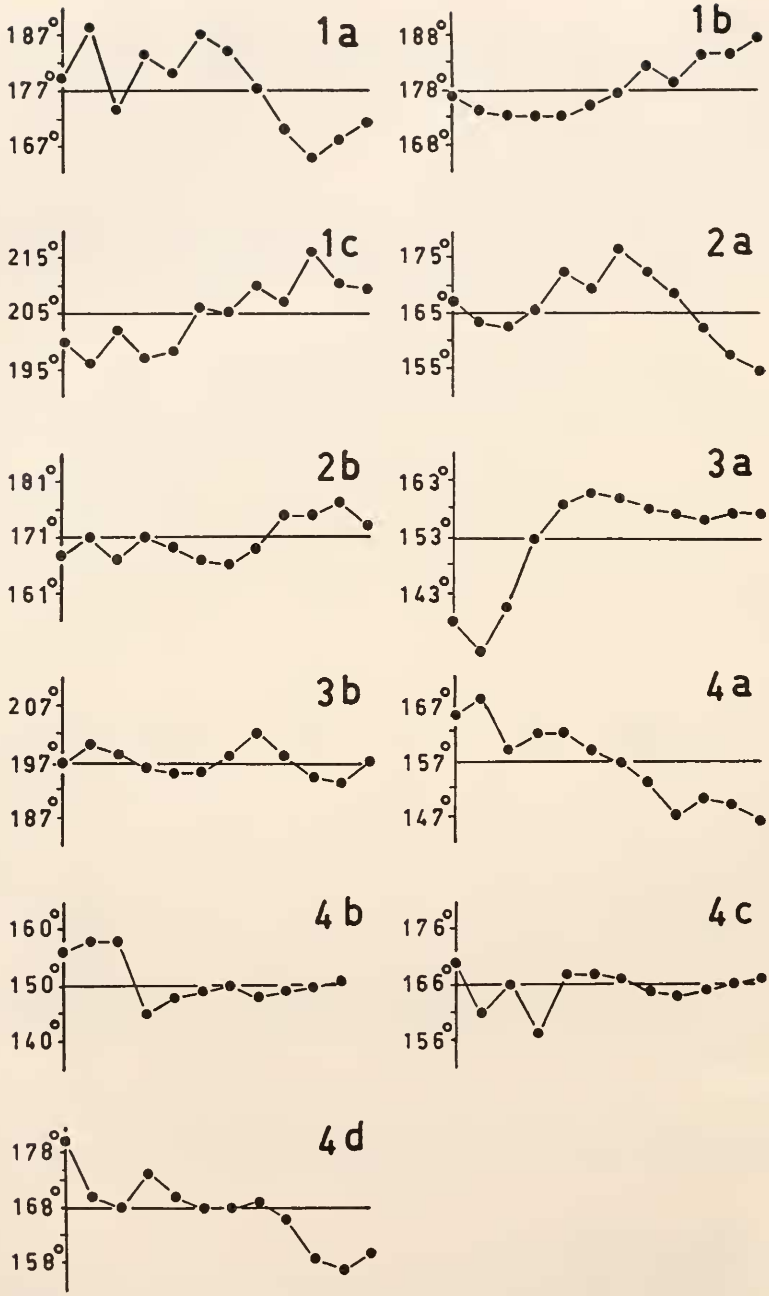

FIGURE 1. The resultant directions (RD) in each experiment. The horizontal line in each graph indicates the value of the resultant direction calculated by means of the accumulated distribution; circles indicate the RD of the single distributions.

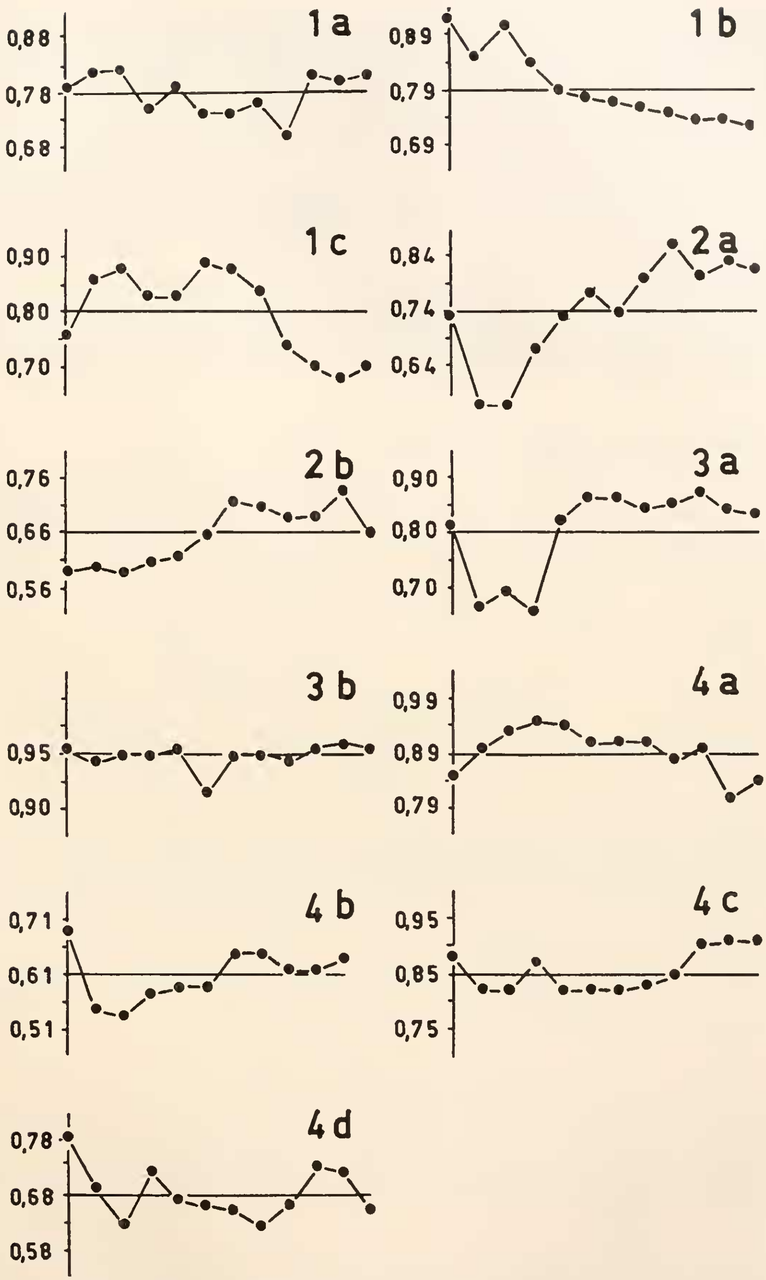

FIGURE 2. The degree of scatter (OR) in each experiment. The horizontal line in each graph indicates the OR calculated by means of the accumulated distribution; circles indicate the OR of the single distributions.

there is an oscillation of 29°, but it seems clear that the animals, during the first three photographs, still showed the positive phototactic behavior which precedes the correct orientation (the lunar azimuth was 134°). It may be noted that the variations of the RD do not seem to follow a fixed pattern. The differences between the RD of the first single distribution and the RD of the accumulated distribution are also shown in Table I. The algebraic average of these differences is less than 1° ($\bar{X} = +0°54' \mp 7°12'$) and consequently we retained, as already stated, the use of the RD of the accumulated distribution as a valid index of the animals' orientation.

The degree of the scatter (Fig. 2) does not vary considerably, nor in any regular pattern. It should also be noted that the OR is always greater than the value of 0.50 which Enright considered the minimal value having significance when calculated for groups of 20 or 30 animals.

2. *The variations of the angle of orientation*

In general the animals seemed to be well oriented, since only in two experiments out of eleven did the RD of the accumulated distribution differ more than 45° from the theoretically expected direction. Moreover, the degree of scatter, as we have seen, was always so small that the distributions must be considered

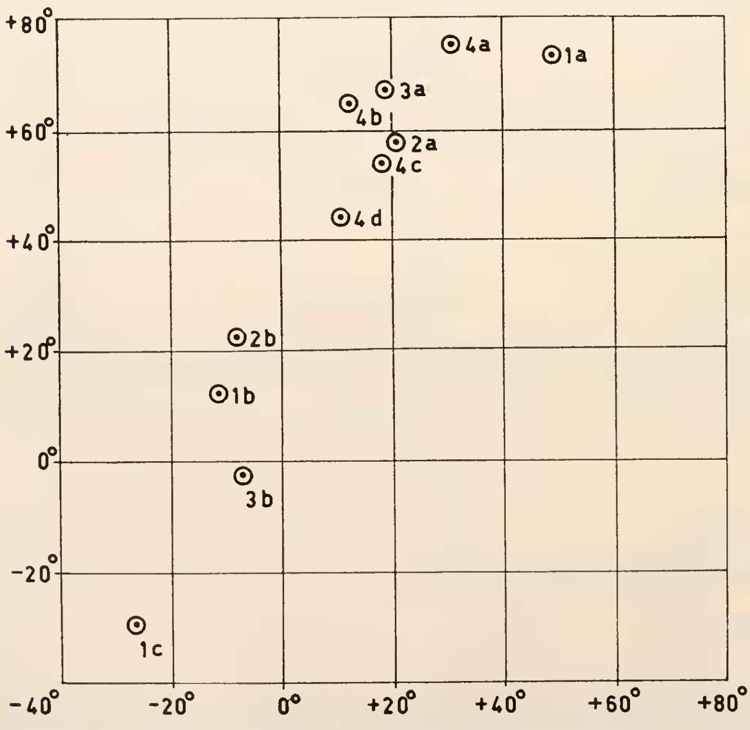

FIGURE 3. Correlation between the theoretical angle of orientation (ordinate) and the observed angle of orientation (abscissa).

significant. In comparison with animals of this same population tested immediately after capture (Papi and Pardi, 1959, p. 587), the results of the current experiments show a stronger tendency to deviate towards south and southeast, a tendency which is generally more marked the nearer the moon is to the east.

A fact of critical importance is whether the angle of orientation with respect to the moon varies in relation to the moon's position or tends instead to oscillate randomly around some other value. Figure 3 represents the correlation between the observed angles and the theoretical angles. The graph indicates a good correlation by inspection. The coefficient of correlation is $r = 0.83$ ($t_r = 4{,}462$; $P < 0.01$). The observed angles of orientation are positive in the first part of the night, tend to become smaller, and then negative, as the moon approaches the west (Table I, col. 9 and Fig. 4). Thus, there is a regular variation in the angles, except for the one night of 3 July. We have not noticed any difference in

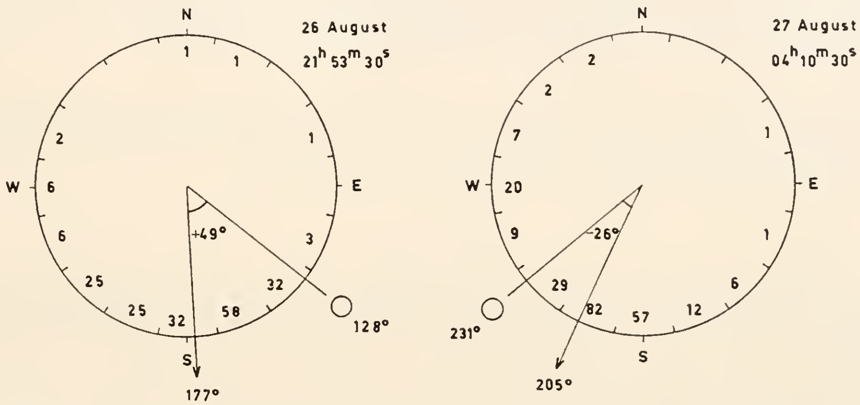

FIGURE 4. An example of the variation of the angle of orientation with moon shown by two lots of animals in the course of the same night. Each number inside the circle shows the number of positions recorded in that sector. The open circles outside represent the moon; the arrows indicate the resultant directions.

the orientation or in the dispersion between animals collected in the morning (series of experiments 1, 3, and 4) and in the afternoon (series of experiments 2).

Discussion

From the results of the above mentioned experiments, we may observe that: (1) the repeated use of the photo-bulb did not produce any noticeable disturbances; (2) the animals continued regularly to vary the angle of orientation, even when they were not exposed to natural light variations resulting from the sunset and from the moonrise.

The stronger tendency to deviate towards the south and southeast, in comparison to our former experiments in which animals were tested immediately after capture, may be attributed to the interruption in the natural light cycle. This could have induced a small shift in the endogenous mechanism of lunar orientation without, however, having arrested its functioning.

We are able, therefore, to confirm the validity of our previous results on *Talitrus saltator* (1953, 1959) and do not find, for this animal, support for Enright's hypothesis that the sunset and/or the moonrise can start (p. 155) "a single cycle of appropriately time-compensated lunar orientation." The hypothesis of an endogenous lunar periodicity, which operates continuously, still seems the most plausible.

The differences between our results and those of Enright could be due to different mechanisms of orientation in the two species. We think it more likely, however, that they are due to the fact that since Enright did not heat the observation chamber nor dehydrate the air inside, the releasing stimuli on certain nights did not attain the necessary threshold. It should be noted that all the experiments with animals "not kept in constant darkness" (Enright's Table II) were made in a single night (6–7 August) and that on the same night even animals "kept in constant darkness" (Table I, last five experiments) oriented with nearly the same degree of precision. It is therefore probable that new experiments on *Orchestoidea* could explain the difference between Enright's results and ours.

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Summary

1. Under the action of appropriate releasing stimuli the amphipod, *Talitrus saltator,* is capable of orienting by the position of the moon in a relatively constant azimuth. This ability still functions after 10 or more hours of captivity in constant darkness.

2. The direction of escape and the degree of scatter are not noticeably influenced by the repeated photo-bulb flashes.

3. Additional evidence supports the hypothesis that the lunar orientation of *Talitrus* is due to a continuously-operating lunar physiological rhythm. The hypothesis of a single-cycle night-time orientation rhythm, put forward for *Orchestoidea corniculata,* does not seem applicable to *Talitrus saltator*.

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